

## Goiter in an Eighteenth-Century Sicilian Mummy

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**ABSTRACT** Goiter is still a frequent pathological condition of the thyroid gland. Goiter consists of an enlargement of the gland caused by several physiopathologic events, the most important of which is deficient intake of iodine. A series of eighteenth-century mummies housed in the church of Santa Maria della Grazia in Comiso includes one individual showing a pathological condition, with a very large swelling in the antero-inferior region of the neck. X-rays showed small scattered radiopaque foci. Routine histology showed a large number of circular follicles immersed in fibrous tissue. Finally, immunohistochemistry revealed a strong reactivity for thyroglobulin. The data confirmed the nature of the tissue as thyroid, and the macroscopic diagnosis is that of thyroid goiter. *Am J Phys Anthropol* 108:427–432, 1999.

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The mummy described in this study is one of 50 mummified individuals housed in the mortuary chapel called the Chapel of the Dead annexed to the church of Santa Maria della Grazia in Comiso (Sicily) at the beginning of the eighteenth century. The individuals are almost all males of different ages, varying from young to old adult, who died in the eighteenth century and the first half of the nineteenth century. The majority are Capuchin friars, and the others are laymen belonging to the third order of the Capuchins. The former are housed in the right wall and the latter in the left wall of the chapel (Fig. 1). Twenty mummies are labeled with the individual's name and date of death, which ranges between 1742 and 1838 (Amadei and Fornaciari, 1996).

All the mummies are natural and had not been submitted to any kind of treatment, either by evisceration or craniotomy. Natural mummification was probably due to the hot, dry climate of Comiso, which is located at the same latitude as Tunis. The processes of dehydration and mummification may also have been aided by the position of the bod-

ies. Until the last century in southern Italy, the bodies of important people were placed for some months in the vaults of churches. The dead were seated on large vases called cantarelle to get rid of cadaveric fluids. They were then disposed horizontally and covered with soil rich in minerals, which completed the processes of dehydration and mummification (Fornaciari and Gamba, 1993; Fornaciari and Capasso, 1996).

All the mummies were found lying in slanting niches of the chapel. They were removed, catalogued, undressed, and submitted to autopsy. The first macroscopic survey showed several kinds of pathology, such as atherosclerosis, splenomegaly, pulmonary fibrosis, and acariasis. In particular, the individual labeled CM 5 showed an enormous enlargement in the antero-inferior region of the neck at the level of the thyroid gland (Fig. 2).

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Fig. 1. An impressive view of the Chapel of the Dead annexed to the church of S. Maria delle Grazie in Comiso. The mummified bodies are lying in the niches of the wall.

For us to confirm the thyroid nature of the enlargement, after X-ray some fragments of the specimen were rehydrated, paraffin-embedded, and processed for hematoxylin-eosin and Van Gieson staining. Finally, immunohistochemistry was performed to reveal the presence of thyroglobulin. Immunohistochemistry has successfully been employed in a number of paleopathological studies, demonstrating that antigenic properties in ancient tissues are preserved and that preservation is related to different mummification processes and body storage conditions (Krypczyk and Tapp, 1986; Fulcheri and Rabino Massa, 1986; Fornaciari and Marchetti, 1986; Fornaciari et al., 1989).

## BACKGROUND

Goiter is a very ancient disease. The most ancient literary references date back to China in the II millennium BC. A detailed description of the disease is reported in *De Architectura* by Vitruvius, written around 25–23 BC. Juvenal and Pliny the Elder (early first century AD) mention endemic goiter in the Alps. In the twelfth century, Ruggero da Parma, a famous *magister* of the Scuola Salernitana, describes, in his *Elettuario Terapeutico*, medical and surgical treatment of the disease. In the thirteenth century, the disease is described as “*bocium fit in gula habitantibus montes*” (“a flower blowing in the neck of highlanders”) in *Compendium Medicinæ*, written by Gilberto Anglico. Three centuries later, Theophrastus Bombastus, well known as Paracelsus, was the first to find a close relationship between endemic goiter and cretinism (Merke, 1960; Brothwell and Sandison, 1967). Moreover, Italian figurative art representing this pathological manifestation has been very rich since the twelfth century (Giampalmo and Fulcheri, 1988).

## MATERIALS AND METHODS

The CM 5 mummy belonged to an anonymous adult male, 1.60 m tall, aged  $25 \pm 10$  years (Todd, 1920; Miles, 1963), wearing linen monastic clothes. The partially preserved soft tissues of the arms, legs, hands, thorax, and abdomen showed a dark yellow skin. The head and feet were almost completely skeletonized, and the mummy had no hair.

During autopsy, which was performed by opening the anterior thorax-abdomen wall after cutting the ribs, the tumefaction of the neck was “surgically” separated from the neck and submitted to X-ray. Small selected fragments were collected to perform histology, histochemistry, and immunohistochemistry.

The specimen removed was white and irregular. It was X-rayed by a portable X GIL S.G. type (70 kV; 7 mA) apparatus produced by Gilardoni (Lecco, Italy) using Du Pont (Wilmington, DE) CRONEX 4 Blue Base film,  $30 \times 40$  cm. Negative films were manually developed using Agfa solution and

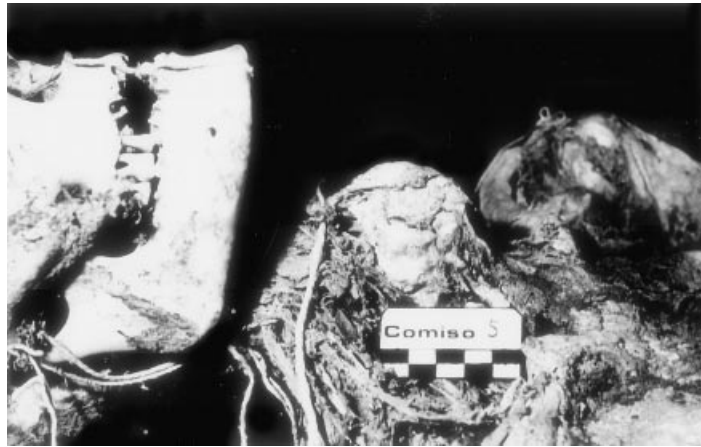


Fig. 2. Lateral view of the CM 5 mummy showing a marked enlargement of the thyroid gland.

fixative. About ten X-rays with anteroposterior and laterolateral projection were performed. Small fragments of the tumefaction were rehydrated using the Sandison method (Sandison, 1955) and processed as routine surgical tissue: they were dehydrated, embedded in paraffin, and then stained with haematoxylin-eosin and Van Gieson.

Histochemistry was performed on previously selected slides using the periodic acid-Schiff (PAS) stain. The immunohistochemical technique was performed on selected sections by applying the immunoperoxidase, avidin-biotin method (Vector, Burlingame, CA). Paraffin sections (5  $\mu$ m thick) were collected from a water bath containing a couple of gelatin sponges, dried, and stored overnight at 37°C. After deparaffinization in xylene and dehydration in absolute alcohol, the tissue sections were incubated in methanol with 0.3% H<sub>2</sub>O<sub>2</sub> to eliminate endogenous peroxidase activity. All the different steps were performed at room temperature. After washing in phosphate-buffered saline (PBS) at pH 7.2, the sections were incubated in 10% normal horse serum. This and all the following antibody solutions were diluted in PBS containing 0.1% bovine serum albumin (BSA). After each incubation, the slides were washed in PBS. The three following incubations were made using 1) primary antibody (monoclonal) vs. thyroglobulin (CIS-DI, Vercelli, Italy), 2) biotinylated horse anti-mouse antibody 1:500 (Vector), and 3) avidin-DH-biotin peroxidase complex (Vector). The reac-

tion was obtained using 0.06% diaminobenzidine (Polysciences, Warrington, PA) and 0.01% H<sub>2</sub>O<sub>2</sub> as substrate. Incubation resulted in a reddish-brown stain for positive cases. After a brief counterstain of the cell nuclei with hematoxylin, the sections were dehydrated in alcohol, cleared in xylene, and mounted. The monoclonal antibodies used were diluted, and each section was covered with 200 ml of solution, corresponding to approximately 2  $\mu$ g of antibody per slide (Hsu et al., 1981).

In each experiment, a positive control (consisting of a tissue for the tested antibody) was simultaneously performed. As a negative control, the same specimen was used omitting in the procedure the primary antibody, using the latter and a second unlabelled antibody. The slides prepared in this way were read and studied by two independent investigators, and, in the evaluation of results, the site of the staining and the quantity of tissue showing specific staining were considered.

## RESULTS

At autopsy, the abdomen and thorax cavities were void of recognizable organs but full of different kinds of materials, such as leaves, fragments of clothes, and paper, evidently introduced there by mice.

The only pathological conditions observed were severe periodontal disease and a small osteoma (ivory exostosis) at the level of the first left premolar of the maxillary.

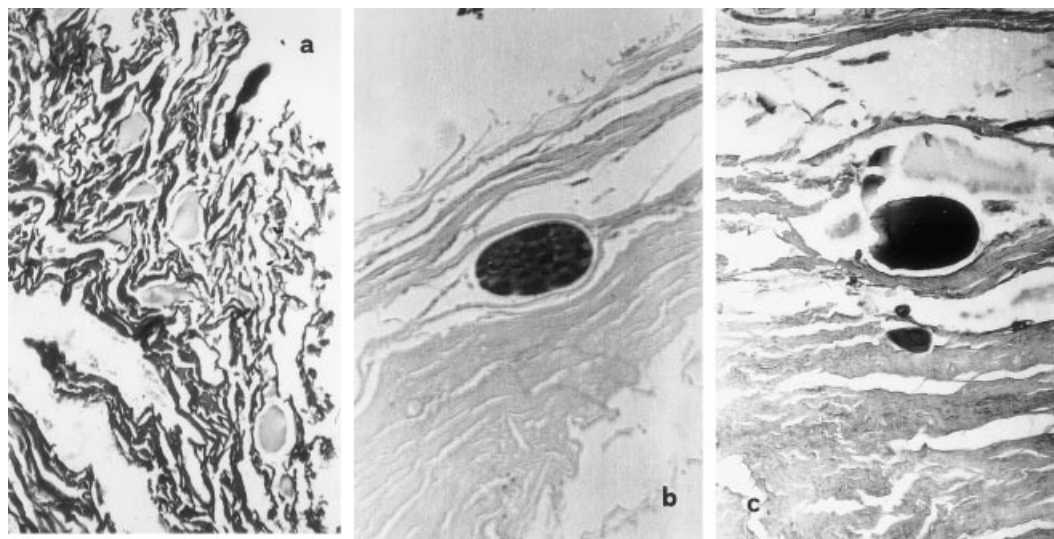


Fig. 3. **a:** Some circular follicles immersed in fibrous tissue evidenced by Van Gieson staining.  $\times 50$ . **b:** Histochemistry of tissue shows one large circular follicle (250  $\mu\text{m}$ ) filled with PAS+ homogenous colloid-like material and immersed in fibrous tissue.  $\times 200$ . **c:** Immunohistochemistry evidenced strong reactivity for thyroglobulin antibody inside the follicles.  $\times 200$ .

The antero-inferior region of the neck showed a very large and well-mummified round subcutaneous tumefaction of the soft tissues ( $6 \times 4 \times 1$  cm) very close to the thyroid cartilage. The organs of the neck were very well preserved, and it was possible to identify easily the larynx, the trachea, and the esophagus, which were macroscopically regular.

On X-ray, the tumefaction showed numerous very small (1–2 mm) radiopaque foci. Once dissected, the foci were revealed to be pathological calcifications. Histological studies showed the presence of numerous semicircular acidophyl clumps surrounded by fibrous tissue, varying from 50–250  $\mu\text{m}$  in the greatest dimension (Fig. 3a). Their homogeneous content was positive on PAS stain (Fig. 3b), and immunohistochemistry revealed strong reactivity for the thyroglobulin antibody (Fig. 3c).

### DISCUSSION

The aim of this study is to establish the tissue nature of the CM 5 neck tumefaction. The dimension and acidophily of the circular formations detected inside the fibrous tissue by Van Gieson staining, their content with homogeneous PAS-positive colloid-like mate-

rial, and finally their strong and specific immunoreactivity for the thyroglobulin antibody clearly demonstrated the thyroid nature of the specimen and the colloid composition of the inside material. The calcifications are due to long-standing hemorrhage since goiter frequently shows regressive phenomena such as hemorrhages, necrosis, or scarring that can explain the presence of dystrophic calcified areas. All the data support the diagnosis of macronodular goiter.

Goiter represents one of the mechanisms by which the gland can compensate for a low intake of iodine or for disorders of iodine biosynthesis. Goiter occurs in endemic or sporadic form. The endemic form occurs mainly in those areas characterized by a low dietary intake of iodine (Andes, Alps, Himalayas); dietary goitrogens such as thiocyanates, fluorides, and calcium may also contribute to endemic goiter. Sporadic goiter is less common than the endemic form, but the mechanisms are not well understood. In both forms, during the early stages, histology is characterized by hypertrophy and hyperplasia of the follicular epithelium, with scant colloid. Later changes show accumulation of colloid and atrophy of the follicular epithelium. A marked secondary enlarge-



ment of the gland follows the massive colloid accumulation and is designated as colloid goiter (Cotran et al., 1994).

The available data do not allow us to establish whether goiter was endemic or not in the area of Comiso. Studies on the contemporary population show no significant occurrences and, in any case, did not employ appropriate epidemiological methods. In contrast, the areas of northern and eastern Sicily are very well studied and documented for endemic goiter (Vigneri, 1988; Regalbuto et al., 1996), which suggests that the disease was uncommon or absent in the area of Comiso. Since the results obtained in our laboratory clearly demonstrate the thyroid nature of the tumefaction and its colloid composition, it is possible to form some reasonable hypotheses regarding the CM 5 case.

Inside the chapel, the CM 5 mummified body was housed in the right wall, which was reserved only for friars, whereas laymen were buried in the left wall. Owing to the friars' habit of often moving away from their birthplaces in the course of their lives, it can be hypothesized that the CM 5 friar came from Sicilian areas outside Comiso where goiter occurs today. The friar likely suffered from the endemic form of disease (Vigneri, personal communication). However, other hypotheses, including the sporadic form of the disease, although less probable, cannot be excluded (Cotran et al., 1994).

A review of paleopathological literature has shown few cases of well-mummified thyroids. Three cases have been reported by Zimmerman, two during his study of the human remains from Nebwenenef's Egyptian tomb (1983) and one from an Eskimo mummy (Zimmerman and Smith, 1975). Fulcheri and Rabino Massa (1986), who investigated the preservation of antigenic properties on mummified tissues by immunohistochemistry, found that only one out of three suspect thyroid samples from Predynastic, Dynastic, and Paleo-Christian mummies were positive for thyroglobulin antibody. As yet, only one case of thyroid disease, recorded and described on the basis of autopsy, has been found in a Peruvian female mummy dated around 94 BC (Gerszten et al.,

1976). In this case, the sex, age, and presence of a marked atherosclerosis supported the diagnosis of hypothyroidism. In the case of Comiso, a similar diagnosis appears to be more difficult because of the absence of typical skeletal features. Furthermore, the sex and the geographical area of the burial were unable to help in the diagnosis. Only modern biomedical techniques have made it possible to diagnose a case of goiter.

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